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Seroprevalence of *Trichinella* infection in domestic swine based on the National Animal Health Monitoring System's 1990 and 1995 swine surveys

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Abstract

Swine sera collected by the US Department of Agriculture's Center for Animal Health Monitoring during 1990 and 1995 was tested for antibodies to *Trichinella spiralis* using an enzyme immunoassay. From a total of 3048 sera collected from lactating sows in 1990, five sera tested positive for a prevalence of 0.16%. From a total of 7987 sera collected from both finishing pigs and gestating sows in 1995, one serum was positive for a prevalence of 0.013%. Responses to questionnaires administered at the time of serum collection showed that seropositive farms had management variables consistent with known risk factors for exposure to trichinae.

Keywords: *Trichinella spiralis*; Trichinellosis; Enzyme immunoassay; Swine; Food safety; Public health

1. Introduction

Trichinella spiralis and related species of *Trichinella* are widely distributed in nature, being found in virtually all warm-blooded carnivores and omnivores. Human infection results when infected meat is ingested raw or without sufficient cooking or processing to kill worms (Currier et al., 1983; Landry et al., 1992; McAuley et al., 1992). Pork and pork products are closely associated with transmission of trichinellosis to humans, however, game meats have recently become a major source of human infection as well (Centers for

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Disease Control, 1991). While the incidence of human infection has declined, group outbreaks still draw attention to hazards associated with eating undercooked pork (Centers for Disease Control, 1991).

The European Union and many other countries inspect swine carcasses for trichinae at slaughter (EEC Directives 77/96/EEC and 84/319/EEC) (EEC, 1977, 1984). The US has no requirements for the inspection of fresh product for trichinae. Instead, the US requires processing to inactivate trichinae in all ready-to-eat pork containing products (Code of Federal Regulations, 1994, Chapter 9) and consumers are advised to cook pork to an internal temperature of 160°F to assure destruction of trichinae.

Reports on the national and regional prevalence of *Trichinella* infection in pigs in the US are limited. Between 1898 and 1906, an infection rate of 1.41% was reported for over eight million swine tested by trichinoscopy (Ransom, 1915). This figure was based on testing only a small sample of tissue and was likely an underestimation of true prevalence. During the period 1933–1937, Schwartz (1940) found 0.95% of approximately 13 000 hogs fed grain or forage to be infected, as determined by digestion of large amounts of tissue. In the same study, 5.7% of 10 500 hogs fed uncooked garbage and 0.55% of 1987 hogs fed cooked garbage were found to be infected. In another study conducted from 1948–1952, Schwartz (1952) found 0.63% of 3031 mid-western grain fed pigs hogs to be infected and 11.21% of 1328 garbage fed hogs from Boston, New York and Philadelphia were found to be infected. Zimmermann and Brandly (1965) found infection rates of 0.12% in 9495 butcher hogs and 0.22% in 6881 breeder hogs between 1961–1965. These same authors found a 2.6% prevalence rate in 5041 hogs fed cooked garbage. In 1966–1970, Zimmermann and Zinter (1971) conducted a national survey, apportioned by regional production. They found an overall prevalence rate of 0.125% in 22 451 hogs, but found 0.51% of 590 garbage fed hogs to be infected. Pigs in New England and NJ had infection rates of 0.73% and 0.58%, respectively in 1985 (Schad et al., 1985a, b). These rates dropped to 0.47% and 0.26%, respectively in 1996 (Gamble et al., 1998). In general, the prevalence of trichinae in pigs has steadily declined over the course of this century; however, prevalence rates vary by geographic region and by husbandry practices.

Despite the apparent sharp decline in prevalence of trichinae in pigs, a stigma still exists regarding this parasite. The purpose of this study was to determine the national prevalence of *Trichinella* in pigs using sera banked from two national swine studies conducted by the USDAs National Animal Health Monitoring System (NAHMS). In addition, we used the NAHMS producer survey to look for associations of seropositivity for trichinae with management practices.

2. Materials and methods

2.1. Swine serum samples

Blood samples were collected during the NAHMS 1990 National Swine Survey and 1995 Grower/Finisher study from randomly selected swine operations in the top hog producing states of the US. In addition, information on management practices were

collected by state and federal Veterinary Medical Officers (VMOs) via an on-farm interview. The 1990 National Swine Survey used a multiple frame sampling technique in 24 states. Producers were randomly selected using multiphasic sampling design in cooperation with the National Agriculture Statistics Service. Selected producers represented 84% of all swine operations and 95% of hogs in the US. For the Swine 1995 Survey, a similar complex survey design was used to randomly select producers from the top 16 swine states. Producers in these states account for 75% of the operations and 91% of hogs in the US. A detailed description of the sampling design, including the generation of weights used for estimation of the national population and the states surveyed, is described elsewhere (NAHMS, 1992; Bush, 1995). In the 1990 study, up to ten samples were collected from farrowing sows. Farrowing rooms were selected using probability proportionate to size methods. In the Swine 1995 study, a maximum of 30 blood samples were collected within selected herds. Fifteen samples were taken from gestating females and the balance were taken from finisher pigs within 30 days of slaughter. Blood samples from sows were distributed evenly among across parities (quotient sampling by age and not probability based sampling). Finishers were not necessarily randomly selected from pens for blood collection. In both surveys, samples and questionnaire data were coded to protect the identity of the producer.

Blood samples collected on-farm were shipped overnight to USDAs National Veterinary Services Laboratory. Serum from the 1990 study were stored in 1 ml aliquots while serum from the Swine 1995 study were stored in 0.4 ml aliquots. Both serum banks were stored frozen at -40°C.

2.2. Enzyme immunoassay

Serum samples were tested for antibodies to *T. spiralis* using the enzyme immunoassay (EIA) as described by Gamble et al. (1983) and Gamble (1996) using an excretory-secretory (ES) antigen. Flat-bottom 96-well microtitration plates (Immulon 2, Costar, Cambridge, MA) were coated with *T. spiralis* (ES) products (0.5 μg/well diluted in 0.1 M carbonate buffer, pH 9.6) by incubating for 1 h at 37°C. Prior to use, plates were washed twice with 200 ul of EIA wash buffer (50 mM Tris buffer (pH 7.4) containing 150 mM sodium chloride, 1.0% Triton X-100 and 5% non-fat dry milk). After washing, the following reagents were then added sequentially and incubated for 30 min at 22°C: (1) 100 μl pig serum diluted 1:100 in wash buffer, (2) 100 μl goat anti-swine IgG (γ-chain specific) (Kirkegaard and Perry, Gaithersburg, MD) diluted 1 : 500 in wash buffer, and (3) 100 μl rabbit anti-goat IgG, conjugated to horseradish peroxidase (Kirkegaard and Perry, Gaithersburg, MD) diluted 1 : 500 in wash buffer. Between all steps, wells were washed three times with 200 μ1 of EIA wash buffer. Following the final wash, 100 μl substrate and chromagen (2,2′-azino-di-[3-ethyl-benzthiazoline] sulfonic acid and hydrogen peroxide) (Kirkegaard and Perry, Gaithersburg, MD) were added and plates read on an automated microplate reader (Molecular Devices, Sunnyvale, CA) at 405 nm. To standardize results, all EIA tests were run until the positive control serum reached an optical density value of 1.00 OD units. A positive cut-off for the EIA was then established as 0.15 × the difference between the reference positive and negative sera as previously described (Gamble, 1996).

2.3. Descriptive analysis

Selected management variables were summarized for suspect and positive serological observations using SAS (SAS, 1996). The variables selected pertained to risk factors for transmission of *Trichinella* infection to swine and included issues of feed and biosecurity (exposure to rodents and wildlife).

3. Results

A total of 3048 samples were tested from 712 operations participating in the 1990 National Swine Survey. A total of 7987 samples, approximately 40% from gestating sows and approximately 60% from grower/finisher pigs, were tested from 286 operations participating in the Swine 1995 study. The distribution of serological results as measured by optical density (OD) are presented in Figs. 1 and 2. For NAHMS 90, the mean value of negative sera in the EIA was 0.076 OD units. A total of five positive sera were identified (Table 1). For NAHMS 95, the mean value of negative sera in the EIA was 0.076 OD units. A single serum sample obtained from a sow was positive (Table 1). The prevalence rates based on these results were 0.16% for 1990 and 0.013% for 1995 (0.030% for sows and 0.0% for grower/finisher pigs). For the 1990 National Swine Survey, taking into account a population of six million sows, there is a 90% confidence limit that the true prevalence is $0.16 \pm 0.12\%$. For the Swine 1995 study, taking into account a population of six million sows, there is a 90% confidence limit that the true prevalence is $0.03 \pm 0.05\%$. For the Swine 1995 study, taking into account a population of 51 million finisher pigs, there is a 95% confidence limit that the true prevalence is less than 0.0625%.

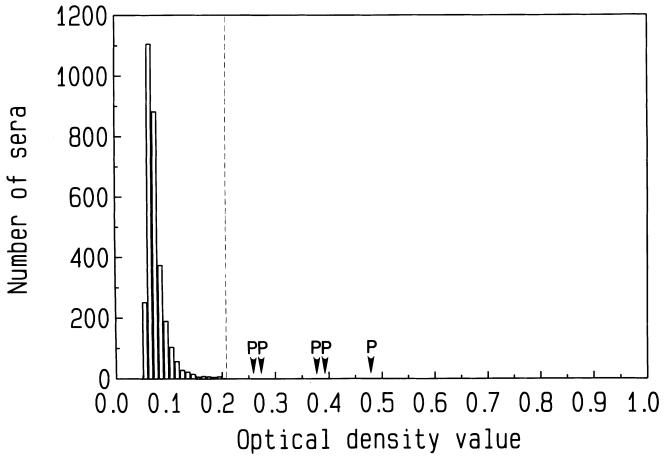

Fig. 1. Distribution of optical density values obtained for 3048 sera from the 1990 National Swine Survey, tested by enzyme immunoassay for antibodies to *Trichinella spiralis*. Positive (P) sera are indicated by arrows. The positive cut-off is indicated by a dashed line.

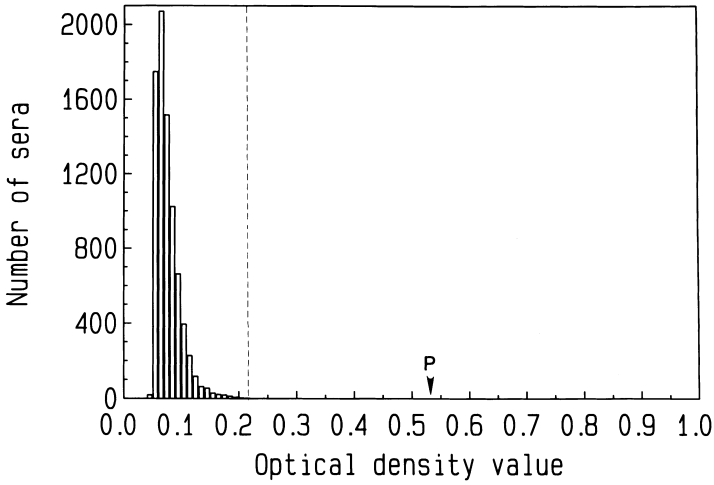

Fig. 2. Distribution of optical density values obtained for 7987 sera from the 1995 National Swine Survey. The positive cut-off is indicated by a dashed line.

The responses to selected farm management variables for the six farms with OD values exceeding the positive cut-off in the ELISA are provided in Table 2. Seropositive pigs were found on farms with total hog numbers ranging from 30 to 2695 animals. For 1990 sera, four of the five seropositive farms raised grower/finisher swine in confinement; one raised grower/finishers on an open lot. Two of the five seropositive premises reported rodents in barns housing grower and finisher hogs. A third farm reported rodents on the premises. Four of the five seropositive farms used cats as a means of rodent control and three farms used rodent bait. None of the farms used rodent traps. Wildlife were routinely seen on all seropositive farms however, only one of the five farms reported outdoor housing of hogs.

For 1995 sera, the single seropositive farm reported housing grower/finisher hogs on outdoor lots. This management practice was recorded for <10% of operations surveyed in Swine 1995, and pigs raised on outdoor lots account for <3% of grower/finisher animals nationally. Rodent control on the seropositive operation included cats and bait, but no traps. No waste food was fed to hogs, but grain was stored in rodent accessible bins.

Table 1
Seropositive samples from NAHMS 1990 and 1995 swine surveys

Survey year	Farm No.[a]	Test result	OD value[b]
1990	1	Positive	0.379
1990	2	Positive	0.266
1990	3	Positive	0.477
1990	4	Positive	0.391
1990	5	Positive	0.266
1995	1	Positive	0.530

[a]Arbitrary identification numbers used to maintain confidentiality.
[b]Optical density values obtained in the enzyme immunoassay.

Table 2
Responses to selected management factors on farms seropositive for *Trichinella spiralis*

Exposure factor	Year/Farm No.						National estimate[a]
	1990/1	1990/2	1990/3	1990/4	1990/5	1995/1	
State	IL	IN	NC	OH	PA	KS	
Pig type	Sow	Sow	Sow	Sow	Sow	Sow	
Perimeter fence	N[b]	N	N	N	N	NA	13.0/NA
Pigs reared in confinement							
Farrowing	Y	Y	N	Y	Y	Y	81.1/56.2
Grow/finish						N	NA/37.9
Rodents seen							
Farrowing	Y	Y	Y	N	N	NA	40.8/NA
Grow/finish	N	Y	Y	N	N	NA	40.0/NA
Wildlife seen on farm							
Racoons	Y	Y	Y	Y	Y	NA	86.5/NA
Opossums	Y	Y	Y	Y	Y	NA	81.7/NA
Foxes	Y	N	Y	N	N	NA	66.0/NA
Skunks	Y	N	Y	Y	Y	NA	81.0/NA
Rodents controlled by							
Cats	Y	Y	Y	N	Y	Y	8.1/68.5
Traps	N	N	N	N	N	N	14.2/13.0
Bait	Y	Y	N	Y	N	Y	78.5/74.0
Feed storage rodent proof	NA	NA	NA	NA	NA	N	NA/36.6
Waste feeder	NA	NA	NA	NA	NA	N	NA/3.9

[a]Estimates for national prevalence (% of farms) answering in the affirmative for each question are given for 1990/1995 NAHMS.
[b]Responses given as yes (Y) or no (N) answers; questions not common to both surveys are indicated by not asked (NA).

4. Discussion

The perception of pork as a carrier of *Trichinella* infection is rooted in a high incidence of the disease in the 1900s and into the 20th century. This perception remains despite a much reduced risk. Human cadaver surveys conducted in the first half of this century showed a prevalence in humans of 13.67% (Hall and Collins, 1937), 17.4% (Nolan and Bozicevich, 1938), 15.4% (Pote, 1939), 22.0% (Most and Helpern, 1941), and 16.1% (Wright et al., 1943). Zimmermann (1967) found a prevalence rate of 2.8% in Iowa in 1961–1965, attributing this to a lower regional incidence in Midwestern pigs as compared with other parts of the US. The national prevalence had declined precipitously to just 4.2% in human diaphragms tested from 1966–1970 (Zimmermann et al., 1973). With age weighting, the prevalence in this latter study was reduced to 2.2% of the population. The decline in human cases was mirrored by a decline in the prevalence in swine, from 1.41% in 1900 to 0.125% in 1966–1970. However, regional infection rates were still as high as 0.58–0.73% in 1985.

In the present study, infection rates in pigs selected from the national swine herd was found to be 0.16% in 1990 and 0.013% in 1995. These rates are lower than recently reported regional rates of 0.26–0.47% in the northeastern US (Gamble et al., 1998), likely

reflecting differences in hog management in the northeast. The 1995 rate of 0.013% reflects a 90% decrease from a national prevalence rate reported 25 years previously (Zimmermann and Zinter, 1971) and likely reflects the continued decline of *Trichinella* infection in US pigs.

This is the first time a national prevalence study for *Trichinella* infection has been performed using a serology test. Previous studies have used digestion of varying amounts of tissue as the method of analysis. Use of serology adds a significant level of sensitivity to the detection of *Trichinella* infected pigs. Previous studies have shown that the EIA test can detect infections with as few as one larvae in several 100 g of tissue (Murrell et al., 1986; Gamble, 1996). In contrast, the digestion method using a five gram sample has a sensitivity of approximately one larvae per gram of tissue (Gamble, 1996). As many as 90% of all natural infections in pigs would be missed using this technique (Schad et al., 1985a, b). No cross reactions have been identified using the EIA test (Gamble et al., 1988). Recently, over 220 000 sera have been tested by EIA with no false positive reactions based on simultaneous digestion testing (Gamble, unpublished). Thus the specificity of EIA is comparable to digestion yet it is more sensitive.

In a recent study of risk associated with *Trichinella* infection and seropositivity (Gamble et al., 1998) we found that statistically significant risk factors included exposure to rodents and wildlife (live and carcasses). Based on the results of the questionnaires completed during the NAHMS 1990 and 1995 surveys, farms with seropositive pigs had management variables consistent with risk factors for trichinae. For 1990 sera, these included presence of wildlife on the farm (5/5), lack of effective rodent control (3/5), and lack of biosecurity (1/5). For 1995 sera, the seropositive farm reported unrestricted access to rodents and wildlife based on outdoor housing of grower/finisher swine. These management variables are consistent with risk factors for exposure of hogs to *T. spiralis*.

The results of this study support a continued decline of *Trichinella* infection in domestic pigs in the US and should be useful to the pork industry in designing education programs to highlight product safety.

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